

Docket No. 1422-0537P

REMARKS

The specification has been amended to provide a cross-reference to the previously filed International Application.

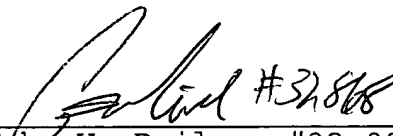
The amendment to the claims is merely to delete improper multiple dependencies and to place the application into better form for examination. Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

Attached hereto is a marked-up version of the changes made to the application by this Amendment.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By  #32,881  
for John W. Bailey, #32,881

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1422-0537P

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Attachment: VERSION WITH MARKINGS TO SHOW CHANGES MADE

(Rev. 02/21/02)

Docket No. 1422-0537P

VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows:

4. (Amended) A DNA comprising at least one wild type FRT sequence and at least one mutant FRT sequence defined in [any one of claims 1 to 3]claim 1.

8. (Amended) A cell which is transformed with the DNA of [any one of claims 4 to 7]claim 4.

13. (Amended) The method according to [any one of claims 9 to 12]claim 9, wherein DNA (a) or DNA (d) is a chromosomal DNA of a cell, and DNA (b) or DNA (e) is a plasmid DNA or a DNA of double-stranded circular DNA virus.

14. (Amended) The method according to [any one of claims 9 to 12]claim 9, wherein DNA (a) or DNA (d) is a chromosomal DNA of a cell, and DNA (b) or DNA (e) has a property for forming a double-stranded circular DNA by intracellular conversion.

15. (Amended) The method according to [any one of claims 9 to 12]claim 9, wherein DNA (a) or DNA (d) is a chromosomal DNA of double-stranded DNA virus, and DNA (b) or DNA (e) is a plasmid DNA or a DNA of double-stranded circular DNA virus.

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16. (Amended) The method according to [any one of claims 9 to 12]claim 9, wherein DNA (a) or DNA (d) is a chromosomal DNA of double-stranded DNA virus, and DNA (b) or DNA (e) has a property of forming a double-stranded circular DNA by intracellular conversion.

18. (Amended) A transgenic animal carrying the DNA of [any one of claims 4 to 7]claim 4 on chromosomes.

19. (Amended) A pharmaceutical comprising the DNA of [any one of claims 4 to 7]claim 4.

(Rev. 11/13/01)

Application No. 10/089,380

- (2) TCTCCAGA (f2151) (SEQ ID NO:3)
- (3) TATCTTGA (f2262) (SEQ ID NO:4) and
- (4) TTTCTGGA (f61) (SEQ ID NO:5)

wherein said mutant FRT sequence is any one of SEQ ID NO[s]S: 2 to 5;--

Please replace the paragraph beginning on page 9, line 19, with the following rewritten paragraph:

--The DNA comprising the mutant FRT sequence of the present invention is a DNA comprising a mutant FRT sequence having a sequence resulting from substitution of nucleotides at middle 8-bp (spacer region) in the following wild type FRT sequence (SEQ ID NO: 1) derived from yeast 2  $\mu$  DNA:

	1 2 3 4 5 6 7 8	
5'-GAAGTTCCTATAC	<u>TTTCTAGA</u>	GAATAGGAACTTC-3'
	spacer region	

with nucleotide sequences selected from the group consisting of the following (1) to (4):

- (1) TCTCTGGA (f2161) (SEQ ID NO:2)
- (2) TCTCCAGA (f2151) (SEQ ID NO:3)
- (3) TATCTTGA (f2262) (SEQ ID NO:4) and
- (4) TTTCTGGA (f61) (SEQ ID NO: 5)

wherein said mutant FRT sequence is any one of SEQ ID NOs: 2 to 5. Since the DNA of the present invention comprises a sequence selected from the group consisting of the items (1) to (4) mentioned above, there are exhibited excellent properties such that in the presence of FLP recombinase, a recombination reaction between two mutant FRT sequences each having an identical sequence to each other is caused, but no recombination reaction with the wild-type FRT sequence is caused. Further, by using the DNA of the present invention, gene replacement can be performed with an even higher efficiency of gene replacement.--

Please replace the paragraph beginning on page 44, line 19, with the following rewritten paragraph:

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--The expression plasmid pEGFP-C1 (4.7 kb, manufactured by CLONTECH) was inserted with DNA sequence encoding mutant green fluorescent protein (GFP). Thereafter, the following synthetic DNA linkers of 18 bp:

5' -GATCTTACTAGTAGGATC-3' (SEQ ID NO:35)

3' -AATGATCATCCTAGAGCT-5' (presented in the 5'-3' direction, SEQ ID NO:36),

which were designed to have a *Bgl*II site at one end and an *Xho*I site at the other end as well as to contain continuous two stop codons in its sequence, were inserted between the *Bgl*II site and *Xho*I site in the multi-cloning site present between the 3'-end of GFP gene and poly(A) sequence on plasmid pEGFP-C1, to give plasmid pEGFP-s.--

Please delete the Original Sequence Listing, filed March 29, 2002, independently numbered pages 1/16 to 16/16. Please insert the attached Substitute Sequence Listing, independently numbered pages 1-7, directly after the abstract.

***IN THE CLAIMS:***

1. (Amended) A DNA comprising a mutant FRT sequence having a sequence resulting from substitution of nucleotides at middle 8-bp (spacer region) in the following wild type FRT sequence (SEQ ID NO: 1) derived from yeast 2  $\mu$  DNA:

5'-GAAGTTCCTATAC	1 2 3 4 5 6 7 8 <u>TTTCTAGA</u> spacer region	GAATAGGAACTTC-3'
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with nucleotide sequences selected from the group consisting of the following (1) to (4):

- (1) TCTCTGGA (f2161) (SEQ ID NO:2)
- (2) TCTCCAGA (f2151) (SEQ ID NO:3)
- (3) TATCTTGA (f2262) (SEQ ID NO:4) and
- (4) TTTCTGGA (f61) (SEQ ID NO:5)

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wherein said mutant FRT sequence is any one of SEQ ID NOS: 2 to 5.

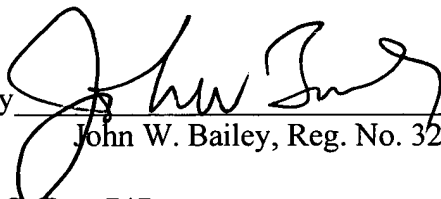
### REMARKS

Enclosed herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a Substitute Sequence Listing to be inserted into the specification as indicated above. The Substitute Sequence Listing in no way introduces new matter into the specification. Also submitted herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a disk copy of the Substitute Sequence Listing. The disk copy of the Substitute Sequence Listing, file "1422-0527P.st25A.txt", is identical to the paper copy, except that it lacks formatting.

The specification and claims have been amended to include the SEQ ID NOS where appropriate. No new matter is introduced by these amendments.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,  
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Attachments:      Disk Copy of Substitute Sequence Listing  
                         Paper Copy of Substitute Sequence Listing  
                         Copy of Notice  
                         Version with Markings Showing Changes Made

(Rev. 03/27/01)

Application No. 10/089,380

**VERSION WITH MARKINGS TO SHOW CHANGES MADE*****IN THE SPECIFICATION:***

The paragraph beginning on page 4, line 14, has been amended as follows:

Concretely, the present invention relates to:

[1] a DNA comprising a mutant FRT sequence having a sequence resulting from substitution of nucleotides at middle 8-bp (spacer region) in the following wild type FRT sequence (SEQ ID NO: 1) derived from yeast 2  $\mu$  DNA:

	1 2 3 4 5 6 7 8	
5'-GAAGTTCCTATAC	<u>TTTCTAGA</u>	GAATAGGAACTTC-3'
	spacer region	

with nucleotide sequences selected from the group consisting of the following (1) to (4):

- (1) TCTCTGGA (f2161) **(SEQ ID NO:2)**
- (2) TCTCCAGA (f2151) **(SEQ ID NO:3)**
- (3) TATCTTGA (f2262) **(SEQ ID NO:4)** and
- (4) TTTCTGGA (f61) **(SEQ ID NO:5)**

wherein said mutant FRT sequence is any one of SEQ ID NO[s]S: 2 to 5;

The paragraph beginning on page 9, line 19, has been amended as follows:

The DNA comprising the mutant FRT sequence of the present invention is a DNA comprising a mutant FRT sequence having a sequence resulting from substitution of nucleotides at middle 8-bp (spacer region) in the following wild type FRT sequence (SEQ ID NO: 1) derived from yeast 2  $\mu$  DNA:

	1 2 3 4 5 6 7 8	
5'-GAAGTTCCTATAC	<u>TTTCTAGA</u>	GAATAGGAACTTC-3'
	spacer region	

with nucleotide sequences selected from the group consisting of the following (1) to (4):

- (1) TCTCTGGA (f2161) **(SEQ ID NO:2)**

(2) TCTCCAGA (f2151) (**SEQ ID NO:3**)

(3) TATCTTGA (f2262) (**SEQ ID NO:4**) and

(4) TTTCTGGA (f61) (**SEQ ID NO: 5**)

wherein said mutant FRT sequence is any one of SEQ ID NOs: 2 to 5. Since the DNA of the present invention comprises a sequence selected from the group consisting of the items (1) to (4) mentioned above, there are exhibited excellent properties such that in the presence of FLP recombinase, a recombination reaction between two mutant FRT sequences each having an identical sequence to each other is caused, but no recombination reaction with the wild-type FRT sequence is caused. Further, by using the DNA of the present invention, gene replacement can be performed with an even higher efficiency of gene replacement.

The paragraph beginning on page 44, line 19, has been amended as follows:

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which were designed to have a *Bgl*II site at one end and an *Xho*I site at the other end as well as to contain continuous two stop codons in its sequence, were inserted between the *Bgl*II site and *Xho*I site in the multi-cloning site present between the 3'-end of GFP gene and poly(A) sequence on plasmid pEGFP-C1, to give plasmid pEGFP-s.

#### **IN THE CLAIMS:**

1. (Amended) A DNA comprising a mutant FRT sequence having a sequence resulting from substitution of nucleotides at middle 8-bp (spacer region) in the following wild type FRT sequence (SEQ ID NO: 1) derived from yeast 2  $\mu$  DNA:



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5'-GAAGTTCCTATAC      12345678  
                         TTTCTAGA      GAATAGGAACTTC-3'  
                         spacer region

with nucleotide sequences selected from the group consisting of the following (1) to (4):

- (1) TCTCTGGA (f2161) **(SEQ ID NO:2)**
- (2) TCTCCAGA (f2151) **(SEQ ID NO:3)**
- (3) TATCTTGA (f2262) **(SEQ ID NO:4)** and
- (4) TTTCTGGA (f61) **(SEQ ID NO:5)**

wherein said mutant FRT sequence is any one of SEQ ID NO[s]S: 2 to 5.